Ultrastructural Modification Along the Glandular Region of the *Diatraea saccharalis* (Lepidoptera: Pyralidae) Silk Gland, at the End of the Larval Stage

Eliane Victoriano; Maria Helena Moreno and Elisa Aparecida Gregório
Centro de Microscopia Eletrônica – Instituto de Biociências de Botucatu- UNESP- Campus de Botucatu, CEP: 18618000,Rubião Júnior, Botucatu, SP, Brasil.

Abstract

Silk glands obtained from *Diatraea saccharalis* (Lepidoptera: Pyralidae) along the last larval instar were conventionally prepared for transmission electron microscopic observation. The first ultrastructural sign of the cellular degenerative process was observed in day 4 after last-instar ecdysis, mainly in the basal cytoplasm of the posterior glandular region. The cells presented many autophagic vacuoles and concentric rough endoplasmic reticulum. The degenerative process extended to the basal cytoplasm of the anterior glandular region later on (day 6 after the last ecdysis). The apical cytoplasm of the posterior region was affected by the degenerative process in day 8 after last-instar ecdysis. The degenerative process of the glandular region in *D. saccharalis* silk gland did not occur synchronously throughout the gland, starting at the posterior glandular region. The sub-cellular alterations are initially concentrated at the basal cytoplasm, the apical cellular region being affected later on.

Keywords: silk gland; degeneration, Lepidoptera; ultrastructure; larvae.

Introduction

The silk gland in Lepidoptera larvae are two paired structures responsible for the production of the silk, used either for the cocoon constitution or to the insect shelter (24, 25, 31). The silk gland is constituted by two distinct regions: the secretory portion, responsible for the synthesis and secretion of the different silk proteins, and the duct, which is often pictured as a mere silk outlet (3, 9). The glandular portion of the silk gland in many Lepidoptera species is described to have two different regions (3, 6, 24, 25). The posterior region is responsible for fibroin secretion, which is the main silk component (3, 18, 22, 24, 25, 31). The anterior glandular region, along their length, secretes the gelatinous silk components, consisting of three types of sericin (3). There are many works on the ultrastructure of the secretory cells of the lepidopteran silk glands, mainly in silkworm (2, 3, 4, 19, 23, 25, 28). During metamorphosis the silk glands in Lepidoptera are either destroyed or change their function (19, 25). Although the general ultrastructural changes concerning the degeneration of the silk glandular cells are described in many insects (8, 9, 13, 19, 24, 25), there are no descriptions of the early ultrastructural features of the silk gland degeneration along their different glandular regions. The sugarcane borer, *Diatraea saccharalis* (Lepidoptera: Pyralidae), is a serious pest of sugarcane and many others crops including maize, sorghum and rice (17). Their silk gland morphology and the ultrastructural features of the secreting cells were previously described: as other Lepidoptera species, the glandular region presents two morphologically distinct regions (29). This gland shows high activity of silk production in the last larval instar (30), when the sugarcane borer is supposed to prepare the nest for the pupation stage. The aim of this study is to describe the early morphological changes of the different glandular cells of the silk gland, at the end of the larval stage, related with the beginning of the silk gland degeneration in the sugarcane borer.

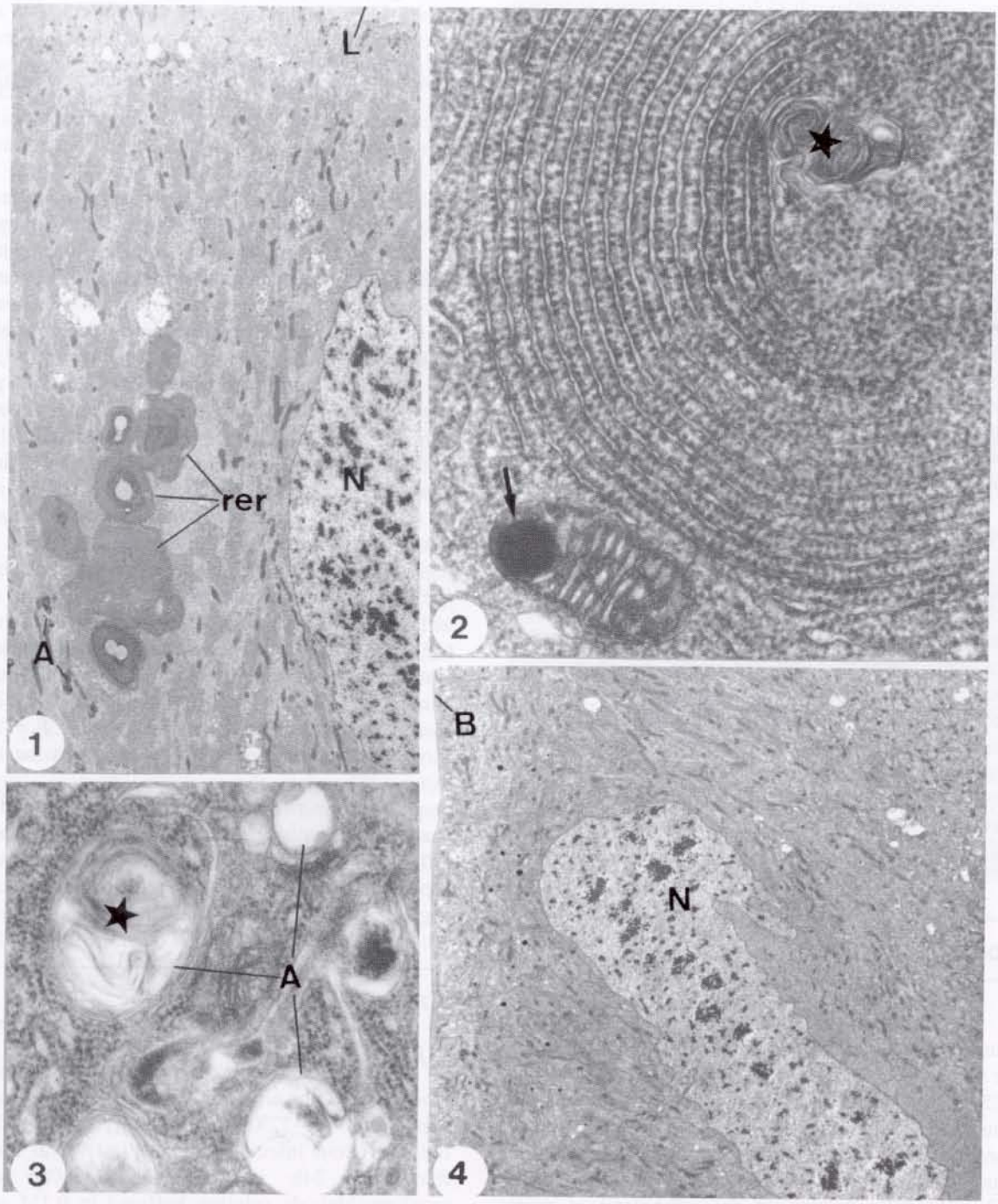
Materials and Methods

The *D. saccharalis* larvae were maintained in the laboratory with artificial diet (12), under controlled temperature (25-27°C) and humidity (70%). Silk glands obtained from insects along the last larval instar were fixed for 24h in 2% glutaraldehyde and 4% paraformaldehyde solution buffered in 0.1M phosphate buffer (pH 7.3) and post-fixed in 1% osmium tetroxide in

the same buffer for 1h. The glands were dehydrated through graded series of acetone, embedded in Araldite resin and examined with a Philips CM 100 transmission electron microscope.

Results and Discussion

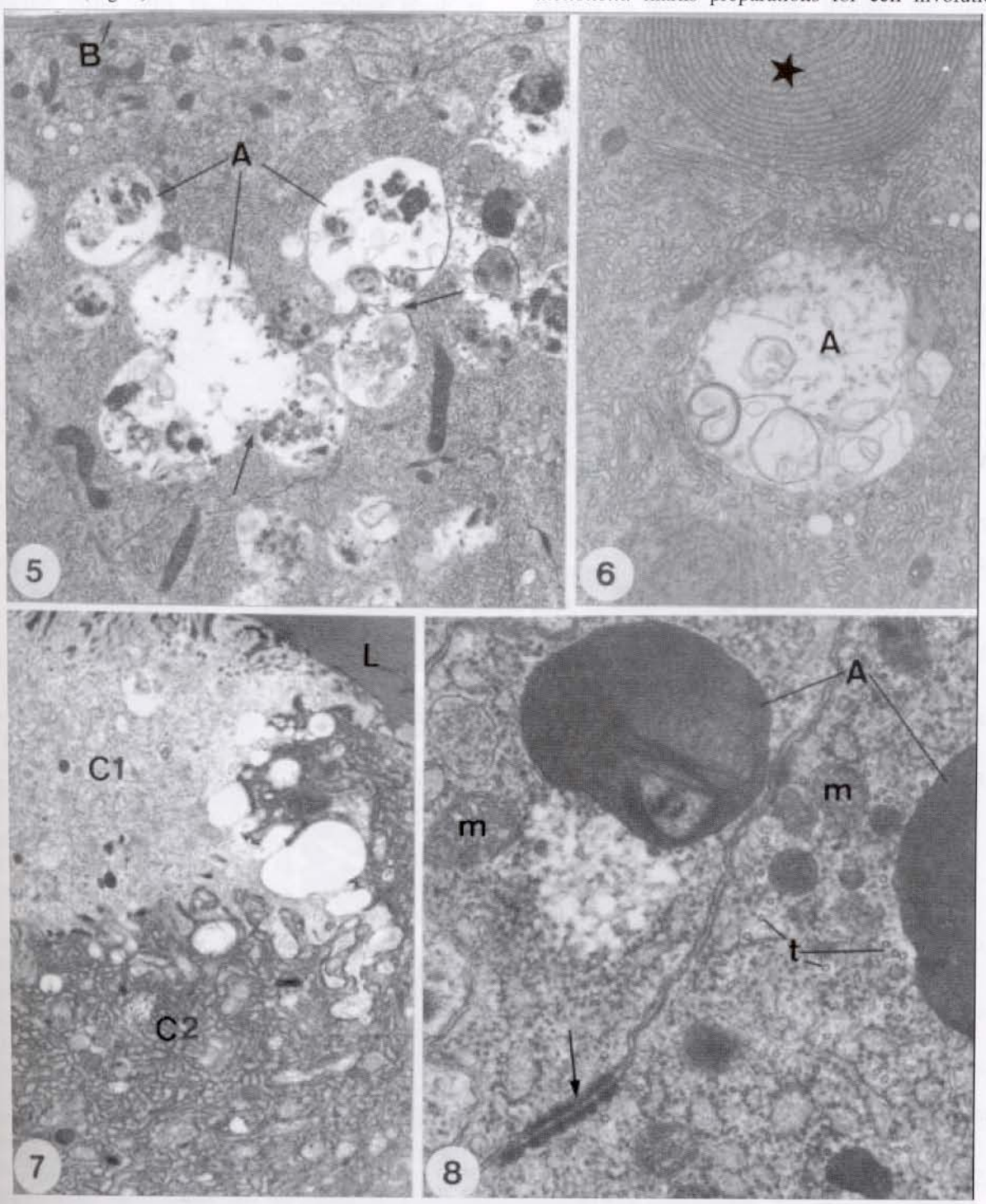
Both the posterior and anterior portions of the glandular region in *D. saccharalis* silk gland exhibit the usual features of silk protein synthesizing cell up to day 3 after last-larval ecdysis, as previously described for this insect (29). On day 4 after last-larval ecdysis the cells of the posterior region present the first signs of degeneration, with the detection of many small autophagic vacuoles (Figs. 1,3) as well as many concentric rough endoplasmic reticulum (RER) (Figs. 1,2); these structures were mainly concentrated in the basal cytoplasm (Fig. 1).



Figures 1-4- Silk gland on day 4 after the last ecdysis. Fig. 1- Posterior Region. Clusters of concentric rough reticulum endoplasmic (rer) and autophagic vacuoles (A); nuclei (N); lumen (L). 2.800X. Fig. 2- Posterior region. Concentric rough endoplasmic reticulum around vacuole with myelin-like structure (✶); mitochondria with electron-dense inclusion (arrow). 57.500X. Fig. 3- Posterior Region. Basal cytoplasm with autophagic vacuoles (A), some containing myelin-like structures (✶). 41.000X. Fig. 4- Anterior Region. Cells with usual features. Nuclei (N), basement lamina (B). 2.800X

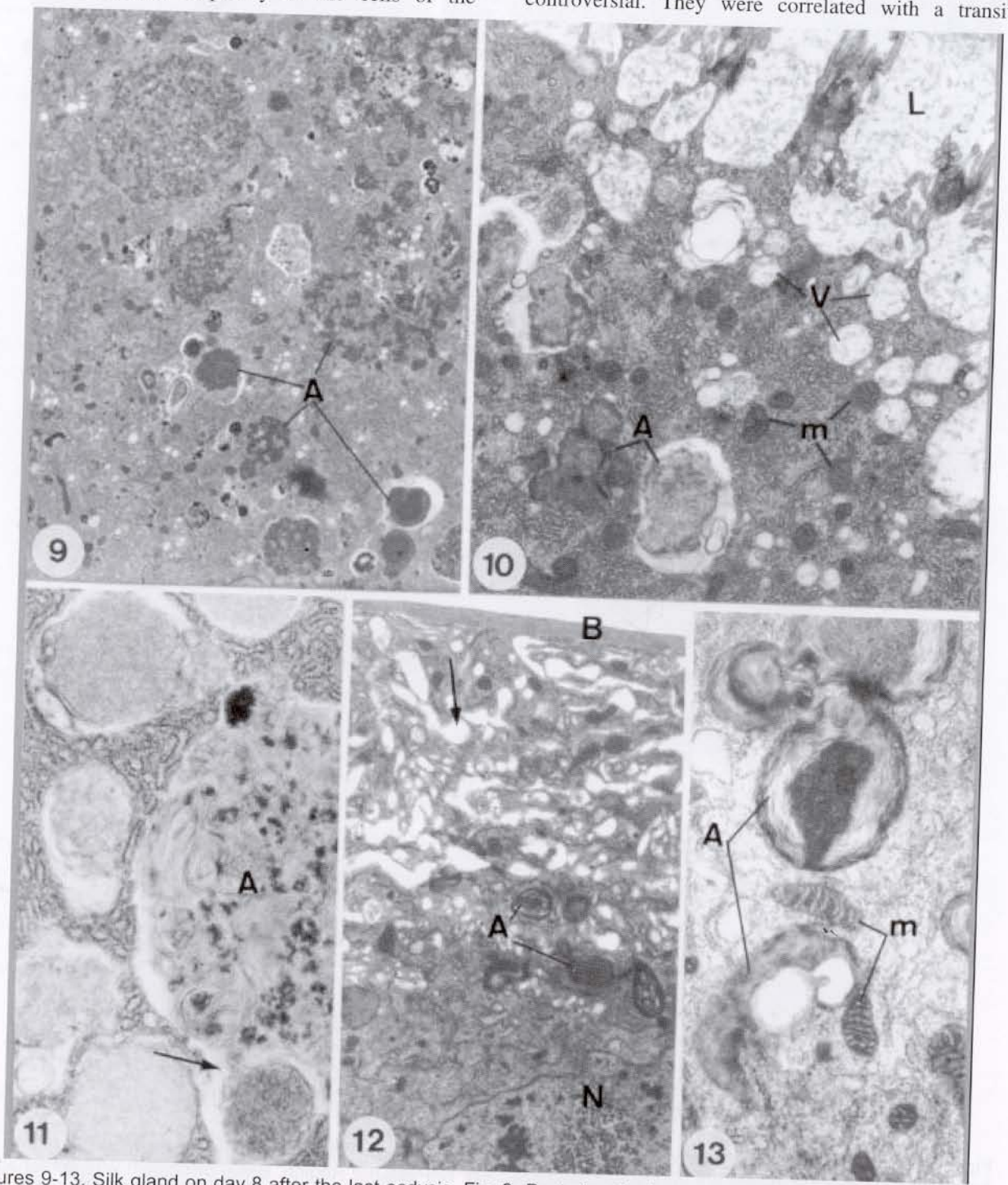
The cells of the anterior glandular region, at the same time, generally present normal subcellular organization (Fig. 4).

The occurrence of some autophagic vacuoles in the cytoplasm of glands of spinning larvae of *Galleria mellonella* marks preparations for cell involution (24).



Figures 5-8. Silk gland on day 6 after the last ecdysis. Fig. 5- Posterior Region. Autophagic vacuoles (A), some in fusing process (arrow); basement lamina (B). 10.250X . Fig. 6- Posterior Region. Basal cytoplasm with autophagic vacuole (A) containing membranous structures; concentric rough reticulum endoplasmic (✶). 14.500X. Fig. 7- Anterior Region. Adjacent cells (C1 and C2) with different ultrastructural features and electron-densities; lumen (L). 7.250X. Fig. 8- Anterior Region. Electron-dense autophagic vacuoles (A), desmosome (arrow); microtubules (t); mitochondria (m). 57.500X.

Early appearance of the autophagic vacuoles, rapid increase in their number and presence of acid phosphatase and probably of other lysosomal enzymes activities were associated with self-destruction mechanism in the silk gland (15). RER in a concentric arrangement has been reported as occurring frequently in the cells of the posterior silk gland at the end of the larval stage (24, 27, 29, 32), but none in the anterior region. Similar compaction of RER in whorls have also been described in resting insect secretory cells (5, 11), vertebrates (26) and plants cells (7). The meaning of these whorls was controversial. They were correlated with a transitory



Figures 9-13. Silk gland on day 8 after the last ecdysis. Fig..9- Posterior Region. Heterogeneous autophagic vacuoles (A) all over the cytoplasm. 4.050X. Fig. 10- Posterior Region. Autophagic vacuoles (A) in the apical cytoplasm; secretory vesicles (V); mitochondria (m); lumen (L). 14.500X. Fig. 11- Posterior Region. Large autophagic vacuoles (A) containing myelin-like structures, some in fusing process (arrow). 21.000X. Fig. 12- Anterior Region. Enlarged basal membrane infoldings (arrow); basement lamina (B); nucleus (N); autophagic vacuoles (A). 7.250X. Fig. 13- Anterior Region. Basal cytoplasm with autophagic vacuoles (A) in fusing process, containing myelin-like structures;

storage form of RER (4), a decrease in their function, as well as with the overproduction of the endoplasmic reticulum (20). In *Galleria mellonella* the transition of silk producing gland cells to the degeneration phase is characterized by dissolution of RER; ribosomes are stripped off and the RER assumes a form of lamellated whorls (24). The appearance of RER whorls correlated with a decrease in the amount of ribosomes that progressively loose their ability to form polysomes (21), suggests that whorls are inactive in exportable protein synthesis, as observed in old fifth instar *Calpodes* larvae (32).

On day 6 after last-larval ecdysis the posterior region cells show a decrease in the number of the apical secretory vacuoles, mitochondria containing electron-dense deposits, discrete increase in the number of both the autophagic vacuoles and the concentric RER (Figs. 5,6). The cells of the anterior region now present some autophagic vacuoles (Fig. 8) and some mitochondria contain electron-dense deposits, as the first signs of degeneration. Apart from the differences between the regions, the ultrastructure very often differs in adjacent cells of the same glandular region (Fig. 7). These differences are sometimes quite considerable. This phenomenon, which has been reported (15), indicates that salivary gland cells, although from one definite organ, remain as units which are to some extent physiologically isolated and independent from each other.

On day 8 after last-larval ecdysis the most conspicuous characteristic of the degenerative process is the large amount of electron-dense autophagic vacuoles, which are present throughout the entire cytoplasm of all the glandular cells in the posterior region (Figs. 9-11). Beside, the cells of the anterior region, at this stage, show enlarged infoldings of the basal plasma membrane (Fig. 12). Some autophagic vacuoles are now detected in the basal cytoplasm of the anterior glandular region (Figs. 12,13), with the same features of the ones described for the posterior region.

Our study showed that the degeneration in the silk gland does not occur synchronously throughout the gland, moving from the posterior to the anterior region of the gland and occurs independently in each cell, as previously described for *Galleria mellonella* (1) and *Manduca sexta* (16). Another finding was that prior to metamorphosing, lysosomes were identified only along the basal surfaces of the cells and then migrate through the cytoplasm and increase in quantity. The increase in lysosomal enzymes from the basal locates and the migration of the lysosomes to the apical region of the cell was a common process for degeneration of many insect cell types (10). Lysosomes probably destroy the machinery of protein synthesis in silk glands during cell death. Our results, as the ones of other authors (10, 14) confirm that the destruction of the cytoplasm is lysosomal; the shift in the lysosomes location suggests changes in intracellular trafficking (10).

Our results showed that the degenerative process of the glandular region in *D. saccharalis* silk gland is asynchronic. It starts at the posterior glandular region and extends to the anterior one. Besides, the sub-cellular alterations are initially concentrated at the basal cytoplasm, the apical portion begin affected later on. The degenerative process may be related with the detection of both the concentric rough endoplasmic reticulum and the autophagic vacuoles.

Acknowledgment

We gratefully acknowledge the Entomological Laboratory of Usina Barra Grande, Lençóis Paulista, S.P., Brasil, for rearing the insects. This research was supported by FAPESP (Proc. 98/03020-0- Ph.D. fellowship).

References

1. Aidels, B.; Lockshin, R.A. & Cullin, A.M. - J. Insect Physiol., 17, p.857 (1971).

2. Akai, H. - Bull. Seric. Exp. Stn., 19, p.375 (1965).

3. Akai, H.; in "Insect ultrastructure", King, R.C., Akai, H., eds., Plenum Press, New York, vol. 2, p. 323 (1984).

4. Blaes, N.; Couble, P. & Prudhomme, J.C. - Cell Tissue Res., 213, p.311 (1980).

5. Bonnanfant-Jais, M.L. - J. Microsc. Biol. Cell., 19, p..295 (1975).

6. Cruz-Landim, C. - An. Acad. Bras. Ciênc., 45, p.565 (1973).

7. Dereuddre, J. - C.R. Acad. Sci. (Paris), 273, p.2239 (1971).

8. Hakim, R.S. - J. Morphol., 149, p.547 (1976).

9. Hakim, R.S. & Kafatos. F.C. - Dev. Biol., 49, p.369 (1976).

10. Halaby, R.; Zakeri, Z. & Lockshin, R.A. - Biochem. Cell. Biol., 72, p.597 (1994).

11. Hecker, H. - Cell Tissue Res., 184, p.321 (1977).

12. Hensley, S.D. & Hammond, A.M. - J. Econ. Entomol., 61, p.1742 (1968).

13. Janda Jr., V. - Acta Entomol. Bohemoslov., 72, p.227 (1975).

14. Jones, H.E. & Bowen, I.D. - Cell Biol. Int., 17, p.305 (1993).

15. Jurand, A. & Pavan, C. - Cell Differ., 4, p.219 (1975).

16- Lockshin, R.A. & Zakeri, Z. - Biochem. Cell Biol., 72, p.589 (1994).

17. Long, W.H. & Hensley, S.D. - Ann. Rev. Entomol., 17, p.149 (1972).

18. Lucas, F. & Rudall, K.M.; in "Comprehensive Biochemistry", Florkin, M., Stota, E.H., eds., Elsevier,, Amsterdam,. vol. 26B, p. 475 (1968).

19. Matsuura, S.; Morimoto, T.; Nagata, S. & Tashiro, Y. - J. Cell. Biol., 38, p.589 (1968).

20. Morimoto, T.; Matsuura, S.; Nagata, S. & Tashiro, Y - J. Cell Biol., 38, p.604 (1968).

21. Prudhomme, J.C. & Couble, P. – Biochimie, 61, p.215 (1979).

22. Prudhomme, J.C.; Couble, P.; Garel, J.P.; Daillie, J.; in "Compreehensive Insect Physiology Biochemistry and Pharmacology", Kerkut, G.A. & Gilbert, L.J., eds., Pergamon Press, New York, vol. 10, p.571 (1985).

23. Sasaki, S. & Tashiro, Y. - J. Cell Biol., 70, p.648 (1976).

24. Sehnal, F. & Akai, H.; in "The Ultrastructure and Functioning of Insect Cells", Akai, H.; King, R.C.; Morohoshi, S., eds., Society for Insect Cells, Tokyo, p.135 (1982)

25. Sehnal, F. & Akai, H. - Int. J. Insect Morphol. Embryol., 10, p.79 (1990).

26. Sehnal, F.; Janda, V. Jr. & Nemec, V. - J. Insect Physiol., 29, p.:237 (1983).

26. Slot, J.N.; Strous, G.J.A. & Geuze, J.J. - J. Cell Biol., 80, p.708 (1979).

27. Tashiro, Y.; Morimoto, T.; Matsuura, S. & Nagata, S. - J. Cell Biol., 38, p.574 (1968).

28. Tashiro, Y.; Shimadzu, T. & Matsuura, S. - Cell Struct. Funct., 1, p.205 (1976).

29. Eliane Victoriano "Morfologia ultra-estrutural da glândula da seda de larva de *Diatraea saccharalis* (Lepidoptera:Pyralidae)" Dissertação de Mestrado, IBB, fevereiro de 1998

30. Victoriano, E. & Gregório, E.A. - Braz. J. Morphol. Sci., 14, p.121 (1997).

31. Wigglesworth, V.B. – "The Principles of Insect Physiology", Chapman & Hall, London (1972).

32. Wiley, M.J. & Lai-Fook, J. - J. Morphol., 144, p.297 (1974).